

An annotated checklist of groundwater Cyclopoida and Harpacticoida (Crustacea, Copepoda) from Romania with notes on their distribution and ecology

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Abstract

Romania, by its position in East-Central Europe has a complex geological history, high landscape heterogeneity and variable climatic conditions, ranging from temperate-continental in the northeast to sub-Mediterranean in the southwest. These conditions have created repeated waves of colonisation of groundwater by copepods, which raise particular interest in this group from a biogeographical perspective. The earliest investigations on groundwater copepods, dating back to the beginning of the 19th century, have resulted in a wealth of information on the richness of this group, making Romania one of the best studied regions from this perspective in Europe. Groundwater copepods in Romania are currently represented by 107 species and subspecies, of which 60 are harpacticoids (56%) and 47 are cyclopoids (43.9%). Of these, 50.5% are strict stygobites (32.7% harpacticoids and 17.7% cyclopoids). Among stygobite copepods 29 species (35 harpacticoids and 19 cyclopoids) are endemic to the country. Almost 86% of the species are single-site endemics (single cave, or single hyporheic or phreatic site) and the rest are restricted in distribution to a single aquifer or hydrographic basin. The aim of the present checklist represents a significant contribution to the knowledge of groundwater copepods in Romania and provides a sound baseline for future comparative faunal studies focused on the affinities and origins of copepods and the analysis of their biogeographical distribution patterns at regional and continental scales.

Keywords

Biodiversity, endemics, Romania, Subterranean habitats

Introduction

Crustaceans are the most diversified group of aquatic invertebrates in groundwater, representing more than 65% of the total groundwater species currently known from Europe (Danielopol et al. 2000; Deharveng et al. 2009; Gibert et al. 2009; Iannella et al. 2020). Amongst them, copepods have been recognised as an important component of the subterranean diversity, the harpacticoids being represented by over 700 stygobites, whereas cyclopoids are represented by ~ 350 species (Iannella et al. 2020).

Copepods are highly diversified in groundwaters and commonly subjected to isolation and allopatric speciation and discontinuous distributions because of vicariance in isolated areas or following ancient drainage patterns (Stoch 1995; Galassi 2001; Galassi et al. 2009; Gibert et al. 2009). They have restricted dispersal capabilities and are limited by the availability of space and the fragmentary nature of groundwater habitats (Galassi 2001; Stoch et al. 2009; Stoch and Galassi 2010). These characteristics make copepods a useful model taxonomic group to test fundamental hypotheses on both drivers shaping current distribution and patterns of species richness in subterranean habitats (Galassi 2001).

In the last decades, several works have been oriented towards explaining copepods high diversity at a continental level and especially in the western and central part of Europe (Eme et al. 2014; Zagmajster et al. 2014; Iannella et al. 2020). This high diversity has been interpreted as a result of traits (e.g., miniaturization, heterochrony) to colonise groundwater, thus undergoing speciation by means of vicariance and subsequent radiation as hypothesized by Stoch (1995). An essential factor contributing to the diversity and distribution of copepods in European groundwaters is related to past and present climatic conditions (Galassi 2001; Eme et al. 2014).

Eastern Europe has a great diversity of groundwater fauna in karst and non-karst regions distributed from the Black Sea to the highest peaks of the Carpathians and Balkan Mountains. This heterogeneous landscape has been shaped by paleogeographic and paleoclimatic events (Decu and Racoviță 1994; Pandourski 2007; Iepure et al. 2015; Iannella et al. 2020). Romania, as a south-eastern country of central Europe, has a distinctive and rich groundwater fauna as compared to the western and the other countries of central Europe (Botoșăneanu 1986; Decu and Racoviță 1994; Negrea and Boitan 2001). Oceanic, western, Mediterranean, euxinic, and temperate-continental taxa compose the groundwater fauna of Romania (Decu and Iliffe 1983; Decu and Racoviță 1994). This area may have worked also as refugium during the Pleistocene glaciations which greatly affected the northern hemisphere; thus, favoring the preservation of ancestral population close to the southern borders of the glaciers covering most part of northern and central Europe (Hewitt 2000; Iepure et al. 2015).

Groundwater crustaceans in Romania are represented by more than 300 taxa (Pleșa 1985; Botoșăneanu 1986) and almost half of them are copepods (Damian-Georgescu 1963, 1964; Botoșăneanu 1986; Decu and Racoviță 1994; Iepure 2007a; Moldovan et al. 2007; data herein). They are reported from all over Romania, with records fragmented and still incomplete throughout the country (Iepure et al. 2015).

Studies of groundwater copepods in Romania have long history dating back to the 1900s when Pierre-Alfred Chappuis started his work on the taxonomy at the Speleological Institute Emil Racovita in Cluj until 1956. Among copepods, the harpacticoids were the subject of his studies in this country; he published more than 160 articles and described more than 70% of the currently known species (Chappuis 1923a, b, 1925, 1928; Delamare-Deboutteville and Rouch 1961; Tăbăcaru 2020). Much of the later knowledge of copepods was derived from the intensive work of Corneliu Pleșa, especially on cyclopoids (between 1956–2000) (Pleșa 1956a, 1956b, 1957a, 1957b, 1958, 1959, 1961, 1963a, 1963b, 1967, 1968, 1969a, 1969b, 1969c, 1971, 1972, 1978, 1985, 1987, 1989; Pleșa and Șerban 1956; Pleșa et al. 1964, 1965, 1996, 1999; Botea and Pleșa 1968; Pleșa and Racoviță 1973; Pleșa and Chintăuan-Mihuț 1996; Pleșa and Buzilă 2000) followed by Damian-Georgescu (Damian and Botoșăneanu 1954; Damian 1955; Damian-Georgescu 1960, 1963, 1964, 1975).

Harpacticoids have been studied mainly by Eugen Șerban (1956) and Doina Zincenco (1967–1970).

The past decade was marked by a renewed interest on the taxonomy of copepods from Romania, resulting in new descriptions of species and new records of species previously known from single or a few sites of this country (Iepure 2007a, b; Iepure and Defaye 2008; Iepure and Oargă 2011; Iepure and Meleg 2011). The last comprehensive survey of copepods at the country level revealed a patchy distribution of copepods primarily in karst areas from northwest and southwest Romania (Moldovan et al. 2001, 2002, 2007, 2011; Iepure 2007a; Meleg et al. 2012, 2014; Iepure et al. 2015; Gaponova 2019). More recently, Moldovan et al. (2020) provided a comprehensive checklist of cave invertebrates comprising also copepods, from Romania.

As part of an ongoing project aimed to revise the systematics of groundwater copepods from Romania, we here assembled an updated checklist of copepods known to date along with some notes on their geographical distribution, habitat preferences and ecology. Our goal is to complement the previous works by including a comprehensive bibliography of copepods in Romanian groundwater and unpublished data.

Materials and methods

Geographic settings

The study area is Romania (centered on 45°N, 25°E) and cover 238,391 km². The country is located in the south-eastern part of central Europe and is bounded by the Carpathian Mountains, the lower course of the Danube (for a length of 1,075 km),

and the Black Sea. The country has a great diversity in topography (altitudinal range from sea level to 2544 m a.s.l.), geological substrates and climate (Fig. 1).

A total of 1.9% (~ 4400 km²) of the exposed land surface of Romania is represented by isolated karstified rocks of Mesozoic ages, strongly influenced by the Neozoic uplift of the Carpathian Mountains which broke the unity of most limestone outcrops (Onac and Cocean 1996; Onac and Constantin 2004). Patches of karst landscape occur in the entire Carpathian chain. The karst units are more developed in the northwest (Apuseni Mountains) and the southwest (Banat Mountains) sections of the Carpathians, totaling 1,762 km². Smaller karst spots are found with the highest concentration in the western part of the Carpathians and the Mehedinți Plateau (25 km²) (Goran 1982; Onac 2000; Onac and Constantin 2004) (Fig. 1). A high underground cave network density exists in the Southern (155.8 m/km²) and the Eastern (76.1 m/km²) (Goran 1983) of the Carpathians. Outside the mountains, there are limestone outcrops in SE Romania, in the Dobrogea Plateau (267 km²).

The Apuseni Mountains (10,750 km²) include the most important karst area of Romania (Goran 1983) (Fig. 1). In terms of landscape characteristics, Apuseni is the most heterogeneous, with large karstified areas of Mesozoic age in the Bihor mas-

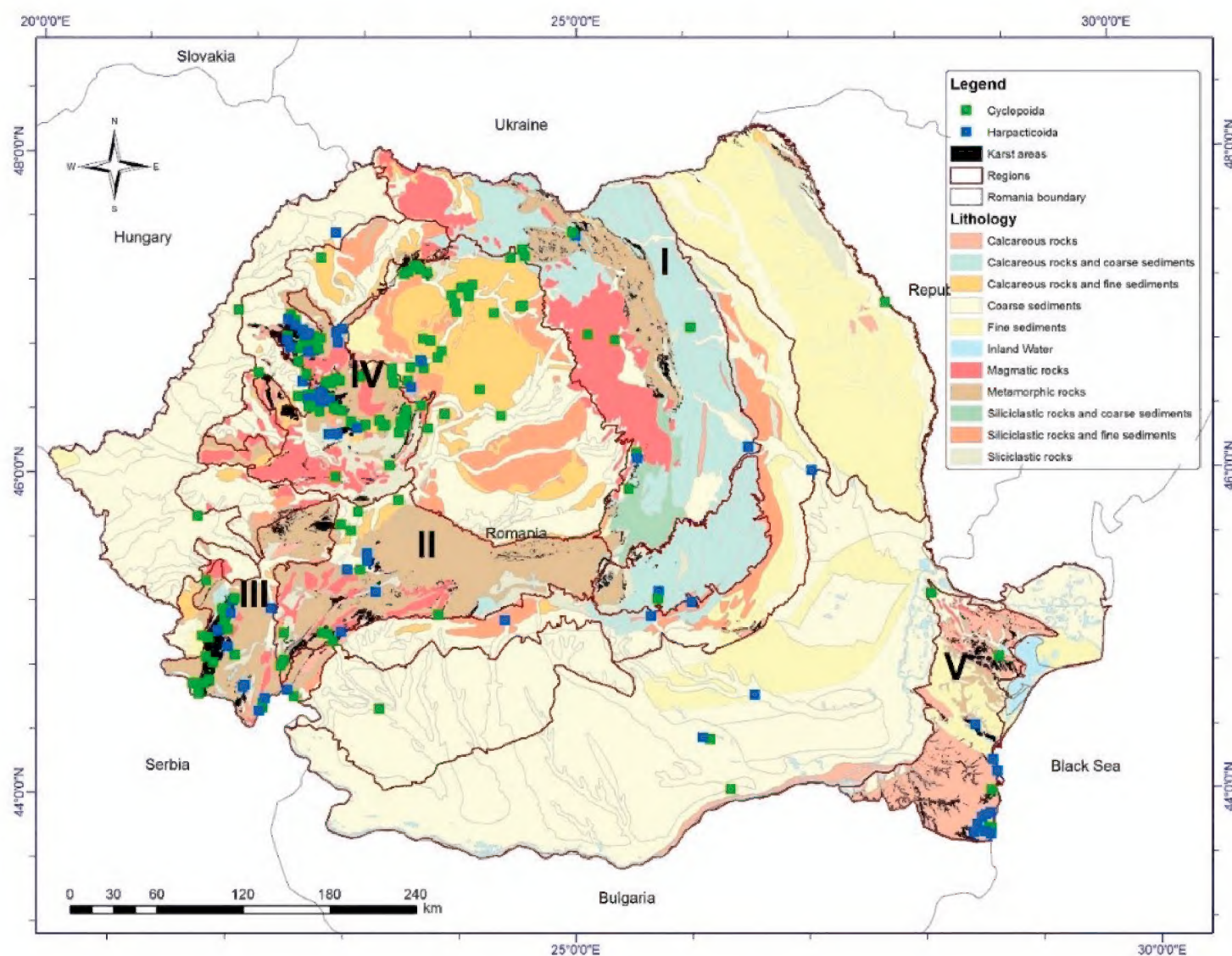


Figure 1. Geological map and distribution of the subterranean localities in Romania with the location of the records of groundwater Cyclopoida (green square) and Harpacticoida (blue square) (I-V: biospeleological provinces after Gibert and Decu 1994).

sif (1000–1200 m a.s.l.), and in the Pădurea Craiului massif in north-east direction (400–800 m a.s.l.). Karstification is less developed in the southeastern Trascău Mountains (below 600 m) (Onac and Cocean 1996; Onac and Constantin 2004). The main rivers have their headwaters in carbonates rocks (e.g., Arieș, Someșul Cald, Crișu Alb, Crișul Repede and Crișul Negru; Orășeanu and Iurkiewicz 2010) and hence large karst aquifers have developed, but porous aquifers are also present in the downstream sectors of some rivers (e.g., Arieș and Someș at 300–400 m a.s.l.).

The Banat karst hosts the largest compact limestone area in Romania (Fig. 1). The karst has a lower density of caves and low underground network development in comparison with the Apuseni Mts. (Goran 1983; Onac and Constantin 2004). At the easternmost part of the Banat and western end of the Carpathian arch between the Motru River and the Danube is located the karst plateau of Mehedinți.

Dobrogea karst comprises a group of low elevation mountains and tablelands between the Danube and the Black Sea (< 500 m a.s.l.) (Fig. 1). The (paleo)karst of Dobrogea occupies less than 1000 km², and is divided into two large sub-regions, Babadag plateau in the north, and the Moesian plateau in the south (Fig. 1). The northern deposits date from the Triassic, while Sarmatian limestone deposits covered by thick layer of loess lies in the south. The karst aquifers of Dobrogea belong to the Danube and Black Sea Basins.

Species list compilation

The Romanian copepods checklist has been assembled by gathering copepod species records available from current literature, species records mentioned in projects reports and unpublished data collected by the author (Sanda Iepure) referred to as *present data*. Data from 485 geo-referenced subterranean localities corresponding to 248 sites were assembled. Copepod species distribution maps were generated using ArcGIS software, version 10.2.2 (ESRI 2011). For this study we created a vector database based on the reviewed literature, which include geographical data (latitude, longitude, and elevation), information about the groundwater habitats including fissured (karstic) and porous (alluvial) aquifers from the entire country and a biological dataset including species presence in all the groundwater habitats for which information was available. The base map includes geology database (Bădescu and Tîrlă 2020).

Subterranean localities included in the dataset account for 107 species and subspecies and records of undescribed and new to science taxa are not included. Sampling in the selected habitats has been constrained by suitable accessibility to the aquifers: caves, wells and hyporheic zone. The methods used for sampling vary from direct water filtering in caves, springs, and wells. The hyporheic zone is sampled by using the Bou-Rouch (Bou and Rouch 1967) and Karaman-Chappuis (Chappuis 1925) methods. Since most of these studies were taxonomic, intensity of sampling was generally low and only in few cases sites were sampled more than twice, especially in caves.

The taxonomy and the recent changes in species names were updated using the Defaye and Dussart (2006), WoRMS database and more recent papers synonymizing families, genera, and species (Kodami et al. 2019). The list of groundwater copepods

including scientific name and authority is presented in alphabetical order, followed by the affinity they show for the groundwater habitats (stygoxenes, stygophiles and stygobites) (cf. Gibert, 1994), habitat, localities from where each species was found, county and reference. The checklist is arranged according to the present taxonomic hierarchy of subfamilies and genera.

Results

In the Suppl. material 1 we listed 107 species of groundwater copepods. While most of the species are recorded from one or a few subterranean habitats, some species are more widely represented in the dataset. Approximately 55% of the sampled sites occur in carbonate rocks and 44% in sedimentary siliceous rocks and a very small portion of records are from sulphidic thermal groundwaters with extreme environmental conditions (Fig. 1).

The elevation of sampling sites ranges from 0 to 1250 m a.s.l., about 81% of sites occurs below 800 m a.s.l. Cyclopoids have been recorded from more than 61% of sites (300 out of a total of 485 records), the remaining ones being harpacticoids (185 records). Ecologically, stygobite copepods are the best represented (54 species representing 51.42% from the total), followed by stygophiles (29 species, 27.2% from the total) and stygoxenes (22 species, representing 20.95% from the total).

Copepods are mostly recorded from caves (113 harpacticoid species and 120 cyclopoid species) and the hyporheic zone (with 27 harpacticoid species and 110 cyclopoid species). The second most diverse habitat for harpacticoids is the tap water (24 species), wells (12 species) and springs (six species); and for cyclopoids, wells (48 species), springs (11) and tap water (10), respectively.

The harpacticoids are the most diverse group in groundwater of Romania, with 60 species (56%) from 184 sites, which belong to three families: Canthocamptidae (with two subfamilies, Canthocamptinae and Morariinae and ten genera), Ameiridae (one subfamily, Ameirinae and two genera) and Parastenocarididae (with five genera). The most frequent species are two stygophiles (*Bryocamptus unisaetosus* and *B. echinatus*) and one stygobite (*Spelaeocamptus spelaeus*) (Fig. 2).

The main representatives harpacticoid copepods were species of the genera *Bryocamptus* (16 species), *Elaphoidella* (10) and *Parastenocaris* (9) (Table 1, Suppl. material 1). *Bryocamptus* has two stygobionts known so far from Romania groundwater; namely, *Bryocamptus* cfr. *baikalensis* from Moanei Cave in Apuseni Mountains and *Bryocamptus balcanicus* from Baile Turcesti spring in Dobrogea.

Elaphoidella is the second more species-rich harpacticoid genus in Romanian groundwater, the majority of species being known from the southern part (Suppl. material 1). The only species found in two very distant karst areas is *Elaphoidella putealis* reported from tap water of Cluj Napoca town and Peștera de la Vadu Crișului, both in northwestern Romania and Buhui Cave in southeast.

Parastenocaris species are mainly known from caves and tap waters (of Cluj Napoca and Bucuresti towns) and only one species is recorded from the interstitial marine sediments close to the Black Sea (Suppl. material 1). The most recent works

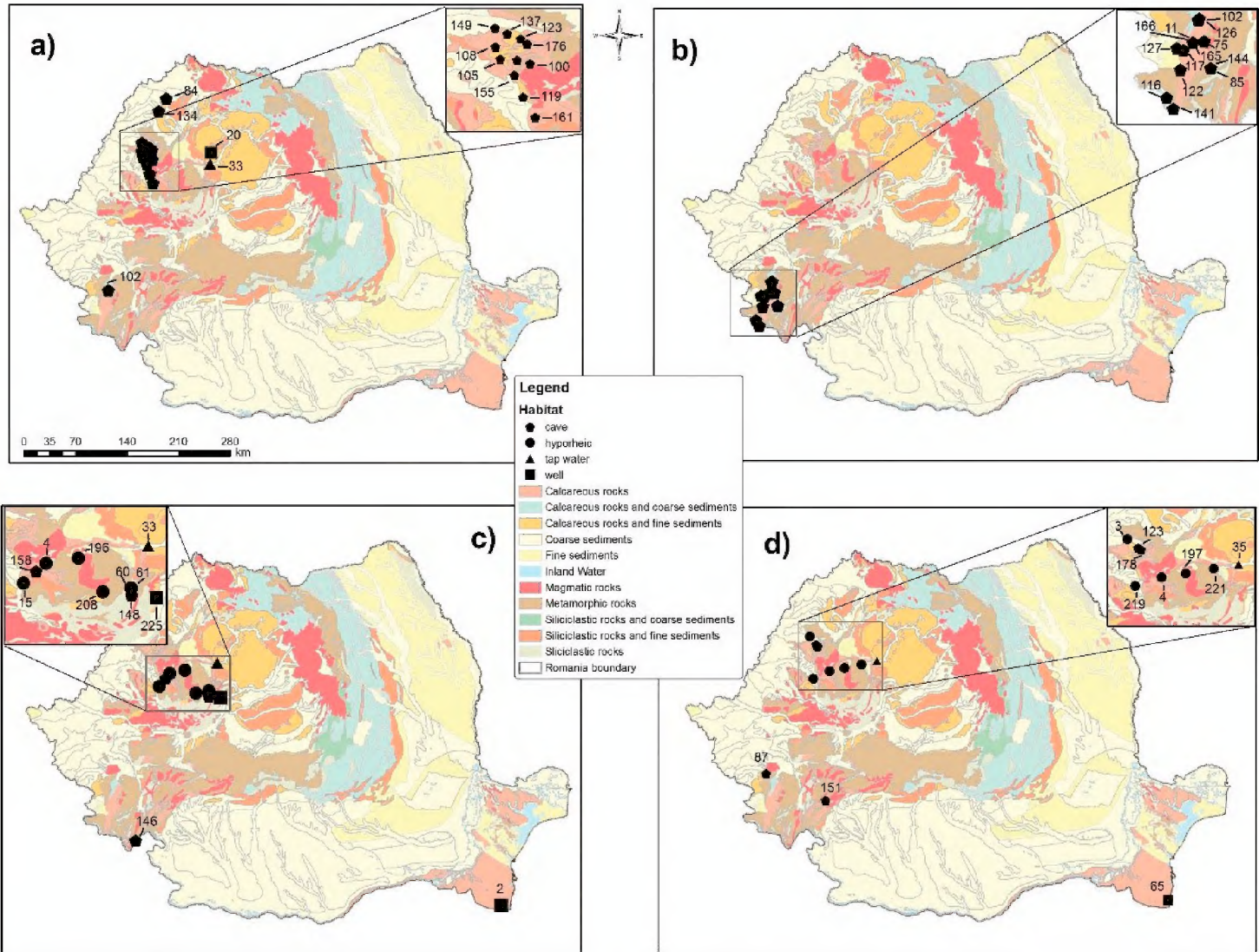


Figure 2. The distribution of the four most abundant stygobite species of copepods in the dataset. **a** Distribution of *Spelaeocamptus spelaeus* (Harpacticoida) in Romania based on 16 occurrences (15 caves and one tap water) **b** Distribution of *Acanthocyclops milotai* (Cyclopoida) in Romania based on 13 occurrences (caves) **c** Distribution of *Diacyclops clandestinus* in Romania based on 13 occurrences (caves, hyporheic, wells, springs, tap water) **d** Distribution of *Acanthocyclops kieferi* in Romania based on 12 occurrences (caves, hyporheic, wells, tap water).

Table 1. Summary of groundwater copepods inventory: number of taxa, ecology, groundwater habitats and endemics.

	Harpacticoida	Cyclopoida
Families	4	2
Subfamilies	4	2
Genera	19	14
Species	59	47
Ecology		
Stygoxenes	8	22
Stygophyles	17	6
Stygobites	35	19
Groundwater habitats		
Caves	113	120
Wells	12	48
Aquifers	24	9
Hyporheic zone	27	110
Springs	6	10
Endemics	18	11

on *Parastenocaris* resurrected the genera *Clujensicaris*, *Nanacaris*, *Proserpinicaris*, and *Stammericaris* established by Jakobi (1972) (Corgosinho et al. 2017).

Cyclopoids with 47 species and subspecies (43.9%) belong to two families, Halicyclopidae (with one genus) and Cyclopidae (with two subfamilies, Eucyclopinae and Cyclopinae and 13 genera). Halicyclopidinae, usually found in brackish waters is represented by one species *Halicyclops rotundipes* reported from interstitial sediments of the Black Sea coast (Fig. 1). Eucyclopinae is represented by species of the genera *Eucyclops* (six species) and *Paracyclops* (three) and were found in all groundwater habitats with a preference for the hyporheic zone of rivers and springs (Damian-Georgescu 1964; Pleșa 1985; Iepure 2007a). Noteworthy to mention is *Eucyclops graeteri scythicus* described from Movile Cave where it inhabits exclusively sulfidic waters (Pleșa 1989; Sârbu et al. 2000; Brad et al. 2021).

The species of *Paracyclops* have been recorded from different types of groundwater habitats in Romania, but mainly from the hyporheic habitat, e.g., *P. imminutus* Kiefer, 1929 and *P. fimbriatus* (Fischer 1853) (Karaytug 1999). *Tropocyclops* is planktonic, frequently found in the eutrophicated lentic warm waters and is rare in groundwater (Dussart and Defaye (2006). So far only one species has been found in Romanian groundwater, *T. prasinus* from two caves and one well from Someșan Plateau, Mehedinți Mountains and Dobrogea, respectively (Damian-Georgescu 1964; Pleșa 1989).

The subfamily Cyclopinae is the main contributor to species diversity among the Cyclopidae and has few genera that successfully colonized groundwaters i.e., *Acanthocyclops* (15 species), *Diacyclops* (nine) and *Speocyclops* (two). All three genera include an important fraction of stygobionts reported from karst and detrital aquifers.

Acanthocyclops has 13 stygobites, two stygoxenes and one stygophile. The stygobiont species of *Acanthocyclops* belong to two distinct lineages: (1) *kieferi*, a highly diversified group in the Mediterranean region including 10 species found in saturated karst (Apuseni and Banat Mountains), and hyporheic zone of rivers (Iepure 2007a, b; Iepure and Defaye 2008; Iepure and Meleg 2011; Iepure and Oargă 2011); and (2) *venustus*, represented so far represented by only one species (Damian-Georgescu 1964; Pleșa 1985; Moldovan et al. 2011) (Fig. 2).

Diacyclops appears highly diversified in the hyporheic zone of Romanian rivers, from which several species at present assigned to the species *D. clandestinus* and species belonging to the *D. languidoides*-group are still to be described. *Speocyclops* has only two species described from Romania, *Speocyclops troglodites* from caves in Apuseni Mountains and *S. lindbergi* from one cave in Banat. *Graeteriella*, a stygobite genus of Cyclopinae widely distributed in groundwater from western continental Europe (Dussart and Defaye 2006), was found so far in only two caves from Apuseni Mountains.

The species of *Cyclops*, a predominantly Palearctic cold-adapted genus and rare in groundwater (Hołyńska and Wyngaard 2019) is represented by only one stygoxene in the hyporheic zone of a river in Someșan Plateau, *Cyclops strenuus* formerly identified by Damian – Georgescu (1964) as *C. rubens*. *Metacyclops* is widespread in tropical and temperate regions, being most prolific in European, African and South American regions, poorly known for North America and Australia. Except for *Metacyclops planus* found in tap water in București, the other two species from this genus, *M. gracilis* and *M. minutus*, are only present in the hyporheic zone.

Discussion

The present checklist provides an updated taxonomy and distribution of copepods in Romanian groundwater. Overall, a total of 107 species and subspecies distributed among four families, five subfamilies and 33 genera of which 54 stygobites are included in this list (Tables 1–2, Suppl. material 1). Considering that stygobiont copepods known from continental groundwater are represented by more than 1000 species/subspecies (Galassi et al. 2009), the Romanian groundwater stygobite copepods listed reach around 5.4%. The two major determinants of biodiversity for the groundwater copepod diversity are likely the same as for many other subterranean groups i.e., productivity and habitat availability but also intensive research in an area and especially in karst (Culver et al. 2003).

The copepod diversity is dominated by Canthocamptidae and Cyclopidae, showing high similarities with those of neighboring ECE regions (Galassi et al. 2009). This also agrees with the results obtained by Eme et al. (2014) and Zagmajster et al. (2010) who observed that the Eastern part of Europe had similarities with the central European area. Furthermore, the Romanian Carpathians appear within the eight biodiversity hotspots of stygobite Crustacea Harpacticoida highlighted beside the Pyrenees (Spain and France), the Jura Massif (France), the Alpine arc (France, Switzerland and Italy) embracing southward the River Po alluvial plain and the Slovenian External Dinarides, the Central Apennines (Italy), the Balkan mountains at the boundary between western Bulgaria and north-west Macedonia, the Dinaric Alps (from Croatia to Albania), the Island of Sardinia and an area in central-northern Europe (including Denmark, the Netherlands and Germany) (Iannella et al. 2020).

Hotspots, referred to as areas of high groundwater biodiversity at the country level are the karst areas of Apuseni (in northwest) and Banat Mountains (in southwest) (Figs 3–5). The exceptional hotspots are three caves in the Apuseni, all located in the northwestern flank of the massif in Pădurea Craiului Mountains, i.e., Vadul Crișului Cave (registering 20 species, of which 15 are harpacticoids and five cyclopoids), Moanei Cave (with 11 species, 10 harpacticoids and one cyclopoid) and Ungurului Cave (with nine species, seven harpacticoids and two cyclopoids) (Table 3). All these hotspot caves have been extensively studied for groundwater fauna and monitored for copepods diversity (Pleșa 1969a; Moldovan et al. 2007).

Copepods seem to be unequally distributed throughout the country, with large *cold spots* especially in non-karst areas or the alpine region in the Carpathian Mountains, which are still poorly explored (Fig. 1). The number of Romanian copepod species is likely to increase in the future since new records are occurring regularly (Moldovan et al. 2011; Meleg et al. 2012; Brad et al. 2020). Furthermore, to establish the taxonomic status of cryptic species, such as those belonging to the *Diacyclops* and some species assigned with uncertainty to *D. clandestinus* – a recurrent situation in groundwater (Stoch 2001), molecular analyses are required.

Groundwater copepods of Romania are divided in two main groups regarding their origin: some families have a direct marine origin, with relatives still living in the primary environment while some others are of more ancient freshwater origin,

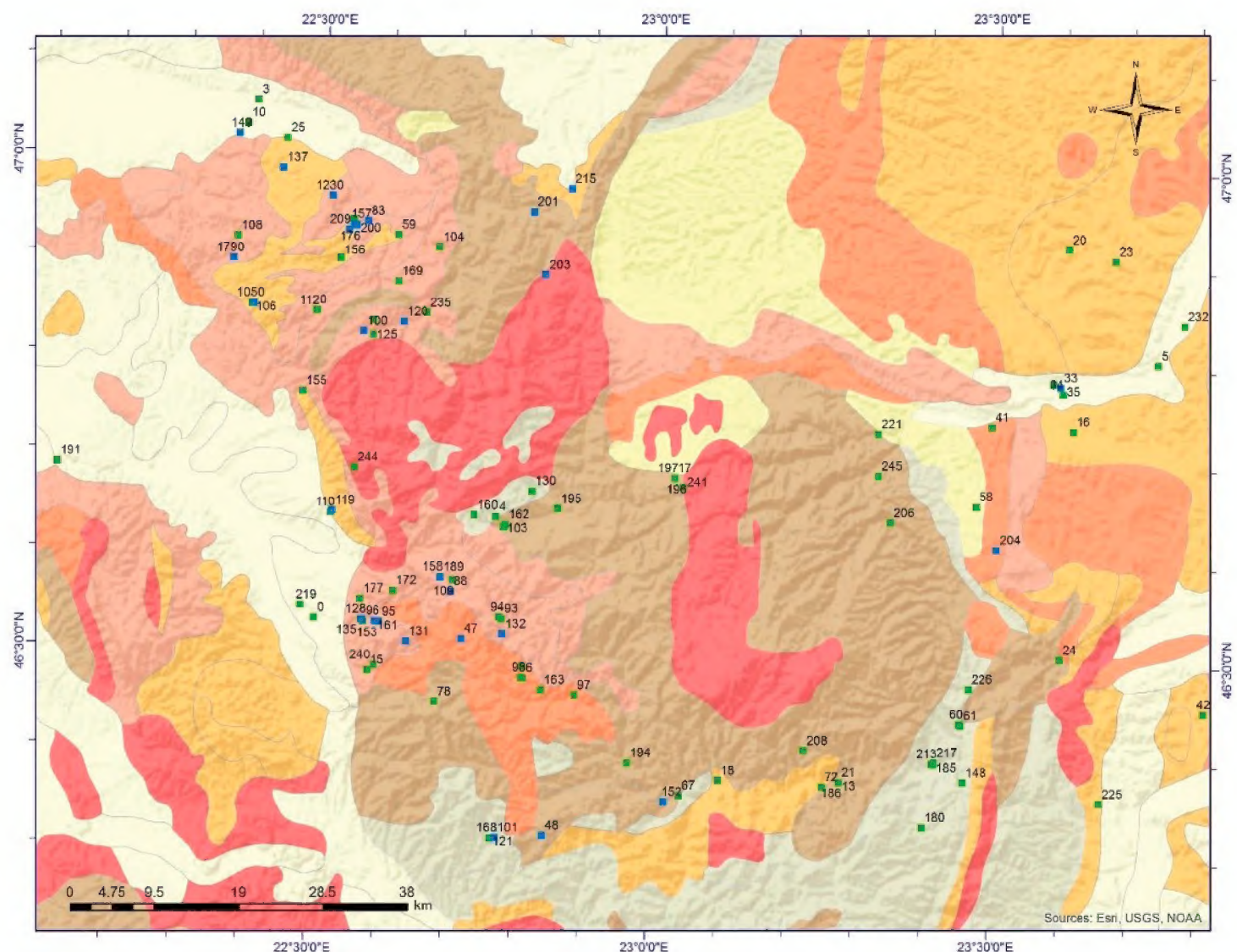


Figure 3. Distribution of Romanian groundwater copepods in Apuseni Mountains (numbers correspond to localities; cf. List of species in Suppl. material 1; green squares – Cyclopoida; blue squares – Harpacticoida).

for which the closest relatives are traceable in surface freshwater or semiterrestrial wet habitats (Boutin and Coineau 1990). To the first group belong mainly the Harpacticoida Ameiridae, with the genera *Nitocrella*, *Nitokra* and *Parapseudoleptomesochra*, and the Cyclopoida Halicyclopidae (Damian-Georgescu 1964; Galassi 2001). Except for *Nitocrella hirta* spotted in the tap water of Cluj-Napoca town, all the rest of the species are distributed along the Black Sea coast (or to less than 150 km from the sea; Damian-Georgescu 1964).

The taxa of freshwater origin are more frequently recorded from Romanian groundwater. Among the Harpacticoida, the most successfully in the subterranean domain are the species of *Elaphoidella*, *Bryocamptus*, *Moraria*, and *Parastenocaris* and the *Eucyclops*, *Acanthocyclops* and *Diacyclops* genera among the Cyclopoida.

Copepod endemism in Romanian groundwaters

The "insular" distribution of the listed endemic copepods is summarized in Table 2. All records from caves and aquifers are directly linked to the fragmented nature of karst aquifers with restricted dispersal potential and with population isolation enhancing speciation (Iepure 2007a; Moldovan et al. 2018).

Table 2. Endemic copepod species from Romanian groundwater habitats.

Species	Localities	Habitat
Copepoda Harpacticoida		
<i>Chappuisius inopinus</i>	București	Tap water
<i>Clujensicaris clujensis</i>	Cluj Napoca, Sura Mare cave	Tap water, cave
<i>Elaphoidella damianae</i>	București	Tap water
<i>Elaphoidella elaphoides</i>	București	Tap water
<i>Elaphoidella gracilis serrulata</i>	București	Tap water
<i>Elaphoidella juxtaputealis</i>	București	Tap water
<i>Elaphoidella romanica</i>	Buhui Cave, Gaura Porcariului Cave	Cave
<i>Nitocrella hirta bucarestiensis</i>	București	Tap water
<i>Parastenocaris chappuisi</i>	Mamaia	Interstitial
<i>Parastenocaris jeanneli</i>	București	Tap water
<i>Parastenocaris karamani brevicaudata</i>	București	Tap water
<i>Parastenocaris latisaetosus</i>	București	Tap water
<i>Parastenocaris minuta</i>	Cluj Napoca	Tap water
<i>Parastenocaris nana</i>	Cluj Napoca	Tap water
<i>Parastenocaris pannonica</i>	București	Tap water
<i>Parastenocaris phreatica</i>	Cetatile Ponorului Cave	Cave
<i>Parastenocaris subterranea</i>	București	Tap water
<i>Parastenocaris uncinatus</i>	București	Tap water
Copepoda Cyclopoida		
<i>Eucyclops graeteri scythicus</i>	Movile Cave	Cave
<i>Acanthocyclops balcanicus bisaetosus</i>	Alun Cave, din Dealul Humpleului Cave, Pepii Cave, Poarta Alunului Cave	Cave
<i>Acanthocyclops milotai</i>	Oase Cave, Buhui Cave, Pauleasa Pothole, Socolovat Cave, Ponor Plopa Cave, Ponor Uscata Cave, Boilor Cave, de sub Carsa Cave, Gaurile lui Miloi II Cave, Padina Matei Cave, Padina Popii Cave, Gaura Haiduceasca Cave, de sub Cetate Cave II, de sub Rol Cave	Cave
<i>Acanthocyclops plesai</i>	Din Fata Rachitii Cave	Cave
<i>Acanthocyclops propinquus</i>	Corbasca Cave, Magura Cave	Cave
<i>Acanthocyclops radevi</i>	Limanu	Well
<i>Acanthocyclops cf. reductus</i>	De dupa Carsa Cave	Cave
<i>Acanthocyclops stygius</i>	Cluj Napoca, Dracoaia Cave, Sighistel Valley	Tap water, cave, hyporheic zone
<i>Acanthocyclops transylvanicus</i>	Cotețul Dobreștilor Cave, Poarta Alunului Cave, Întorsuri Cave Ciur Izbuc Cave, Ungurului Cave, Dobos Cave	Cave
<i>Speocyclops lindbergi</i>	Hotilor de la Baile Herculane Cave	Cave

The total number of endemic groundwater copepods is relatively low, 29 out of a total of 105 (representing 27.62%; Table 2). The greatest percentage of endemics is found among the harpacticoids with slightly more endemics (18 species totaling 17.15% of the total of copepod species) than cyclopoids (11 species, summing 10.48%). However, this number is likely to be underestimated, since recent ecological surveys in caves and the hyporheic zone across the Apuseni Mountains and Someșan Plateau, point to several endemic species new to science that are waiting formal taxonomic description (Meleg et al. 2012; SI, unpublished data). The endemism rate for copepods mirrors that observed for the ECE region, with only Bulgaria having a slightly similar number of endemics, whereas neighboring countries such as Serbia,

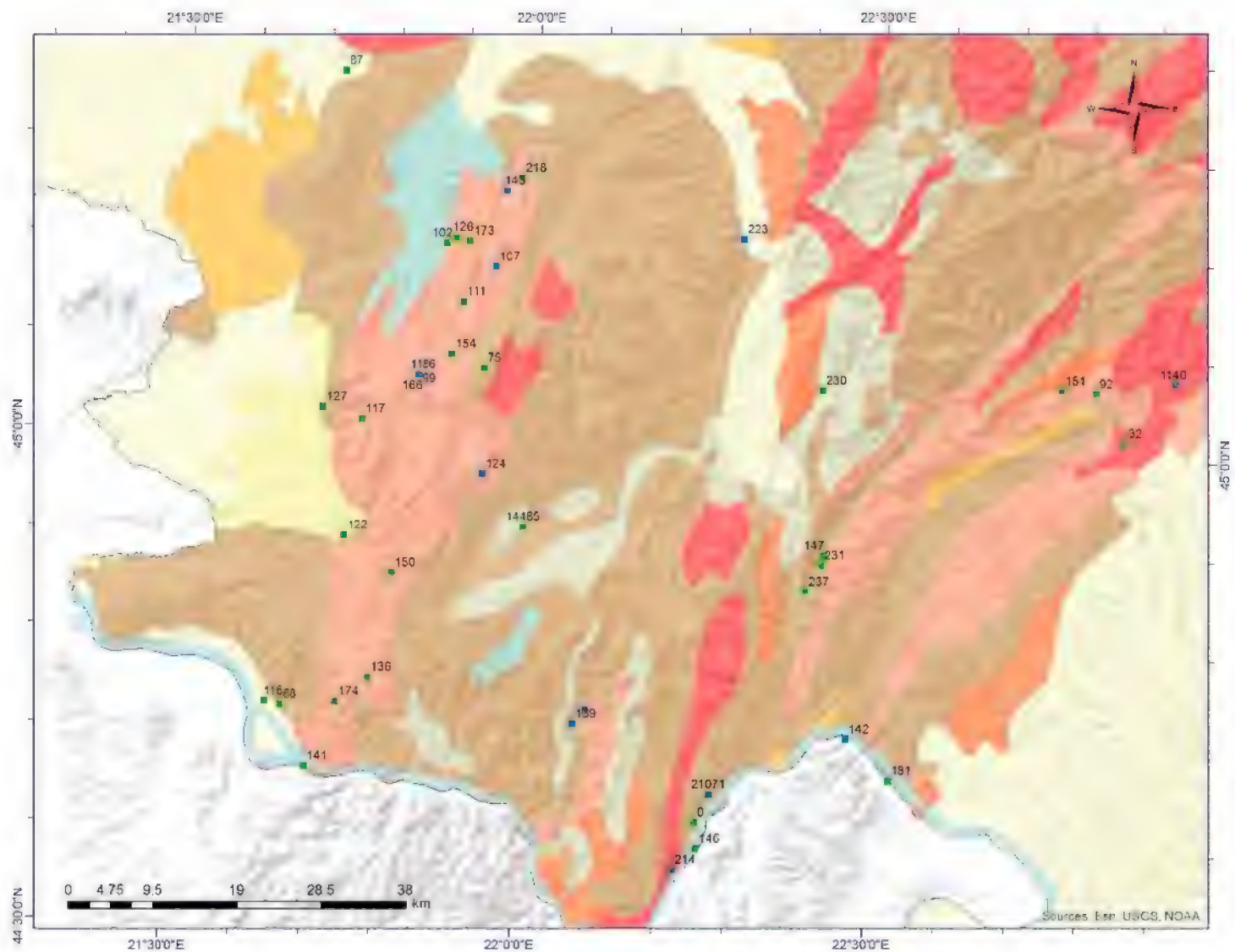


Figure 4. Distribution of Romanian groundwater copepods in Banat (numbers correspond to localities; cf. List of species – Suppl. material 1).

Ukraine and Hungary having significantly lower numbers (Decu and Juberthie 1994). This is likely due to low sampling effort rather than a real reflection of the endemics in groundwater habitats.

The hotspot localities of endemic species in Romania remain the tap water in București (12 endemics out of a total of 19 species) and Cluj Napoca (four out of a total of seven species) described by Chappuis (1923a) (Table 3). There is a high percentage of single-site endemics in Romanian groundwater, 20 species (representing 68.96% of the total endemic species) with only 9 species occurring in more than one locality. However, the endemics with multiple locations are mostly from small areas with restricted distribution to the same aquifer or hydrographic basin. One exception is represented by *Acanthocyclops stygius* recorded from the Someș aquifer and several caves in Apuseni Mountains (> 200 km) and *Clujensicaris clujensis* from the Someș aquifer and a cave in Șureanu Mountains (> 350 km apart) (see Suppl. material 1).

Among harpacticoids, the genus with the highest number of endemics is *Parastenocaris*. Ecologically, *Parastenocaris* species have a preference for interstitial waters but the species in Romania, including the endemics, are all documented from caves (usually

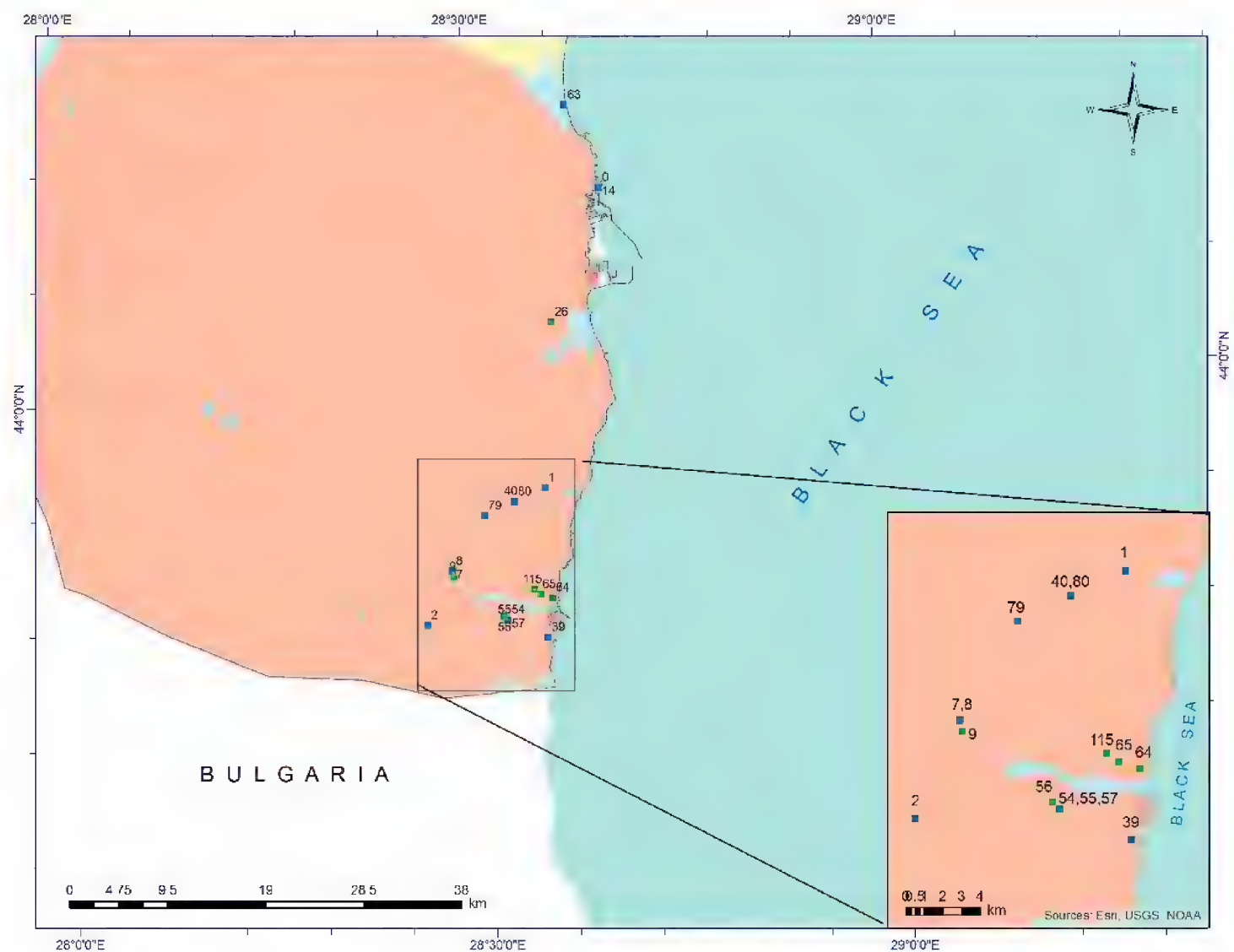


Figure 5. Distribution of Romanian groundwater copepods in Dobrogea (numbers correspond to localities; cf. List of species – Suppl. material 1).

Table 3. Taxonomic summary of cave copepods biodiversity hotspots (with * stygobite species).

Pestera de la Vadul Crisului

HARPACTICOIDA

- Attheyella crassa*
- Bryocamptus caucasicus*
- Bryocamptus dacicus**
- Bryocamptus spinulosus*
- Bryocamptus tatrensis*
- Bryocamptus typhlops*
- Bryocamptus vej dovskyi*
- Maraenobiotus brucei carpathicus*
- Maraenobiotus vej dovskyi vej dovskyi*
- Moraria (Moraria) poppei*
- Moraria brevipes*
- Elaphoidella putealis**
- Pesceus schmeili*

*Spelaeocamptus spelaeus**

CYCLOPOIDA

Eucyclops serrulatus serrulatus

Paracyclops fimbriatus

Diacyclops bisetosus

Megacyclops viridis

*Speocyclops troglodites**

Pestera Moanei

HARPACTICOIDA

Attheyella wierzejskii

Bryocamptus cfr. *baikalensis*

Bryocamptus bispinosus

Bryocamptus dacicus

Bryocamptus echinatus

Bryocamptus minutus

Bryocamptus spinulosus

Bryocamptus tatrensis

*Bryocamptus unisaetosus**

Pesceus schmeili

CYCLOPOIDA

Paracyclops fimbriatus

Pestera Ungurului

HARPACTICOIDA

Bryocamptus caucasicus

Bryocamptus tatrensis

Bryocamptus typhlops

Bryocamptus zschokkei

Maraenobiotus brucei carpathicus

Pesceus schmeili

*Spelaeocamptus spelaeus**

CYCLOPOIDA

*Acanthocyclops transylvanicus**

Paracyclops fimbriatus

unsaturated epikarst) and tap water (phreatic habitat). The exception is *P. chappuisi*, which was found in interstitial waters close to the Black Sea coast.

The second most diverse endemic harpacticoid genus was *Elaphoidella*, with five endemic species out of a total of 10. *Elaphoidella* is cosmopolitan in distribution, usually inhabiting freshwaters and semi-terrestrial habitats. The other harpacticoid genera have usually two or three stygobites endemic species.

Among Cyclopoida the most diverse genera hosting endemics is *Acanthocyclops* with eight endemics out of a total of 15 (Iepure 2007a). All the endemic *Acanthocyclops* are cave dwellers known from the Apuseni Mountains and Banat and belong to the

kieferi-group (Iepure 2007; Iepure and Defaye 2008; Iepure and Meleg 2011; Iepure and Oargă 2011; Moldovan et al. 2020). The *kieferi*-species complex is distributed mainly in south-eastern Europe and the Mediterranean region and probably they are remnants of a Tertiary relict fauna (Pandourski 2007).

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Supplementary material I

Checklist of groundwater Copepoda (Cyclopoida, Harpacticoida) from Romania

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Data type: occurrences, species ecology

Explanation note: Updated checklist of groundwater Copepoda (Cyclopoida, Harpacticoida) from Romania with their occurrence in the sampling sites

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